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Cell envelope of *Neisseria gonorrhoeae* *A comparative study with* Escherichia coli

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Summary

The cell envelope of *Neisseria gonorrhoeae* was studied and compared to that of *Escherichia coli*. Outer membranes (OM) from both species were isolated by an identical method, and subjected to biochemical analysis.

Differences in OM structure were sought that might explain the dissimilarity in OM permeability of these two species. The most pronounced difference appeared to reside in the OM proteins as judged by gel electrophoresis. Moreover, gonococcal OM proteins appeared to be more hydrophilic than those of *E. coli*.

Introduction

Unlike enterobacteria, *Neisseria gonorrhoeae* is normally highly susceptible to antibacterial agents. Several workers have shown that enterobacteria such as *Escherichia coli* possess a barrier against the penetration of antibacterial agents. This barrier has been traced to the outer membrane of the cell envelope (Boman, Nordström, and Normark, 1974; Gustafsson, Nordström, and Normark, 1973; Leive, 1965). It has been claimed that the outer membrane lipopolysaccharides (LPS) are responsible for preventing penetration by drugs (Boman, Jonsson, Monner, Normark, and Bloom, 1971; Gustafsson and others, 1973; Monner, Jonsson, and Boman, 1971; Tamaki and Matsuhashi, 1973). However, it has recently been suggested that hydrophilic areas exist through hydrophilic portions of outer membrane proteins (Inouye, 1974). The number and size of such hypothetical pores should determine the penetrating ability of exogenous substances. The aim of this communication is to provide a biochemical and physiological explanation for the marked difference in antibiotic resistance between *N. gonorrhoeae* and *E. coli*. Part of this work has been published elsewhere (Wolf-Watz, Elmros, Normark, and Bloom, 1975) with a full description of the methods used.

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Findings and discussion

Like *E. coli* the cell envelope of *N. gonorrhoeae* contains an outer membrane (OM) outside the peptidoglycan layer. However, in contrast to *E. coli*, this structure in *N. gonorrhoeae* appears more loosely associated to the peptidoglycan. In *E. coli* the OM is anchored by a lipoprotein bridge covalently bound to the peptide side chain of the peptidoglycan (Braun and Rehn, 1969). When the peptidoglycan prepared from *N. gonorrhoeae* was subjected to chemical analysis no evidence was obtained for such a lipoprotein bridge. The peptidoglycan contained only glucosamine, muramic acid, glutamic acid, alanine, and *meso*-diaminopimelic acid in the molar ratio 1:1:1:2:1.

It has been suggested that the OM of *N. gonorrhoeae* is more permeable than that of *E. coli* and other enterobacteria (Wolf-Watz and others, 1975). This hypothesis is in agreement with our findings that *N. gonorrhoeae* shows an exceedingly high uptake and cytoplasmic concentration of the basic dye gentian violet (Table). This dye does not penetrate the envelope of wild type *E. coli* K-12 and can be measured in only low amounts in the envelope fraction.

TABLE *Total uptake and separation of gentian violet-containing cell material*

Species	*Uptake of gentian violet*		
	Total uptake (Per cent.)	*Relative amount*	
		Envelope	*Cytoplasm*
E. coli	22	95	0
N. gonorrhoeae	89	59	52

Total uptake of gentian violet in *N. gonorrhoeae* strain 82409/55, colony type 4 and *E. coli* strain D21.
For experimental details see Gustafsson and others (1973) and Wolf-Watz and others (1975).

The marked difference in permeability between *E. coli* and *N. gonorrhoeae* suggests a fundamental difference in the biochemical structure of their OM. We have therefore isolated and characterized the OM of *N. gonorrhoeae*. A method, originally worked out for *E. coli* (Wolf-Watz, Normark, and Bloom, 1973), which yields large quantities of relatively pure OM, was employed. The method is based on lysozyme and ethylenediaminetetra-acetic acid treatment of plasmolyzed cells which causes a substantial release of OM. The released OM fragments can easily be recovered by a low speed centrifugation step after lowering the pH to 5·0. Isopyknic sucrose density gradient centrifugation was chosen to establish the homogeneity of our preparation. When OM of *N. gonorrhoeae* was subjected to this treatment only one band with a buoyant density of 1·25 g./cm^3. could be detected (Wolf-Watz, and others, 1975). No material corresponding to cytoplasmic membrane was observed. In our hands no significant difference in OM density exists between *E. coli* and *N. gonorrhoeae* (Wolf-Watz and others, 1973, 1975).

At present only two enzymatic activities have been traced to the OM of *E. coli* and *S. typhimurium*, namely phospholipase A and lyso-phospholipase (Osborn, Gander, Parisi, and Carson, 1972; Wolf-Watz and others, 1973). These activities have therefore been used as specific OM markers. OM prepared from *N. gonorrhoeae* shows a 6-fold increase in specific activity of these lipases as compared to a total gonococcal membrane fraction. No lipase activity was found in the cytoplasmic membrane of *N. gonorrhoeae* (Wolf-Watz and others, 1975). Using the same assay conditions, the specific activity of gonococcal OM lipases was five times lower than the corresponding activity of *E. coli* (0·13 versus 0·80 nmole/min./mg. protein) (Wolf-Watz and others, 1973, 1975).

Our gonococcal OM fraction shows a 6 to 8-fold decrease in typical cytoplasmic membrane markers (succinate dehydrogenase and D-lactate dehydrogenase) when compared to a total membrane fraction. In the electron microscope our OM fraction appears as triple-layered membrane fragments (8–9 nm thick) (Fig. 1). The enzymatic data and the fact that only one density band appears strongly suggest that these trilaminar structures are OM, relatively free of cytoplasmic membrane contamination.

OM of enterobacteria is known to be rich in proteins, LPS, and phospholipids (Osborn and others, 1972; Schnaitman, 1970). This was also found to be true for *N. gonorrhoeae* (Johnston and Gotschlich, 1974; Wolf-Watz and others, 1975).

It is well known that certain permeable mutants of *E. coli* exhibit mutational lesions in the LPS moiety of the OM (Boman and others, 1974; Tamaki

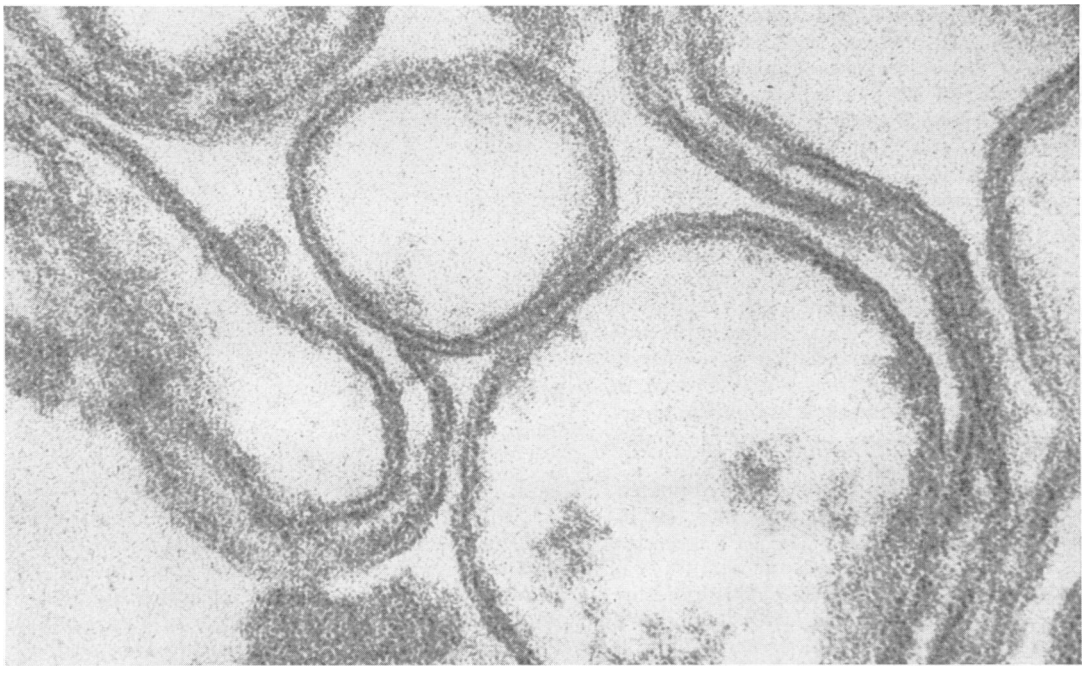

FIG. 1 *Electron micrograph of OM fraction isolated from* N. gonorrhoeae, *colony type* 4. *Notice typical trilaminar configuration of vesicular structures visualized.* ×252,000

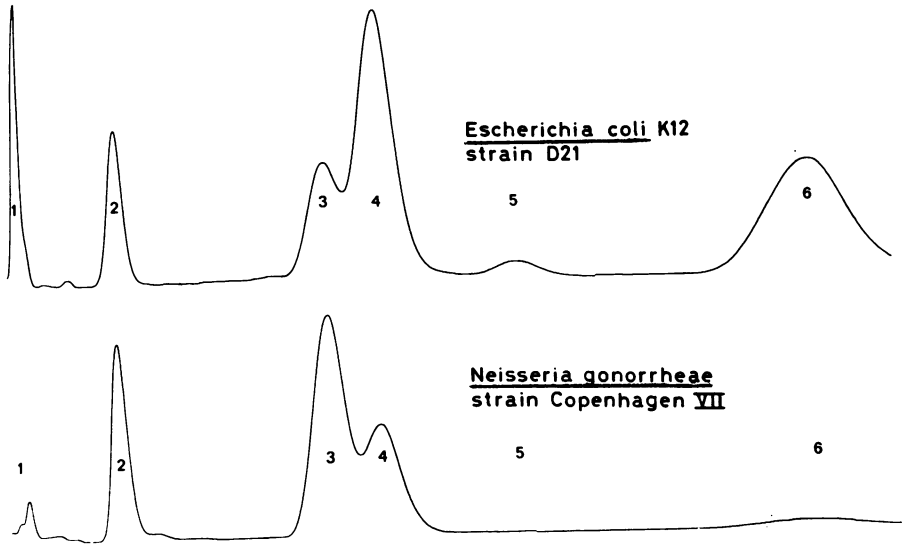

FIG. 2 *Gas chromatograms of lipopolysaccharide carbohydrates isolated from* E. coli *K*-12 *and* N. gonorrhoeae, *colony type 4. Experimental conditions described by Wolf-Watz and others* (1975) *Peak* 1, *rhamnose*: *Peak* 2, *xylose (internal standard); Peak* 3, *galactose, Peak* 4, *glucose; Peak* 5, *unknown, probably degradation product of heptose; Peak* 6, *heptose*

and Matsuhashi, 1973). A gradual loss of LPS carbohydrates has been shown gradually to increase the permeability of the cells (Gustafsson and others, 1973). With gas chromatography, the LPS of both *E. coli* and *N. gonorrhoeae* was found to contain glucose, galactose, and heptose (Fig. 2). In addition it is known that ketodeoxyoctonoic acid and glucosamine are present in LPS of both species (Heath and Ghalambor, 1963; Boman and Monner, 1975; Stead, Main, Ward, and Watt, 1975).

In contrast to Stead and others (1975), we find relatively low amounts of heptose in the LPS of *N. gonorrhoeae*. It is possible that in our preparatory procedure heptose has been partially degraded. However, when the same hydrolysis condition was applied to *E. coli* LPS, a ten times higher amount of heptose was found (Fig. 2). This relative difference may be explained either by a variation in sensitivity of heptose during hydrolysis or by a difference in the true molar ratio of heptose. It should be noted that heptose deficient mutants of *E. coli* are highly permeable (Boman and others, 1974).

All Gram-negative bacteria so far examined possess an endotoxin (Milner, Rudbach, and Ribi, 1971). It appears that the lipid A part of the LPS molecule constitutes this factor (Milner and others, 1971). A low toxic potential of *N. gonorrhoeae* LPS is indicated by the lack of fulminant cases with gonococcal septicaemia. We find that N. *gonorrhoeae* lipid A contains three fatty acids, with retention times corresponding to β-hydroxymyristic acid, lauric acid, and stearic acid. Interestingly, myristic acid—a known constituent of *E. coli* lipid A—is lacking in gonococcal lipid A (Fig. 3). On the other hand, the latter seems to contain stearic acid, a fatty acid totally lacking in *E. coli* lipid A (Boman

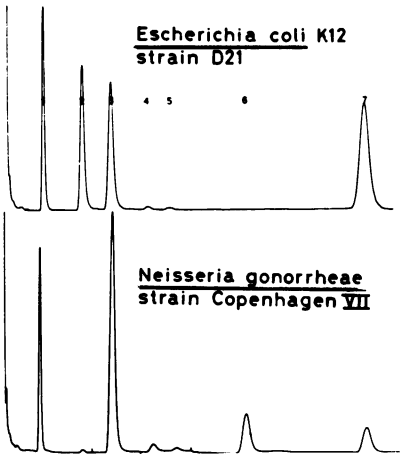

FIG. 3 *Gaschromatograms of lipid A fatty acids prepared from* E. coli K-12 *and* N. gonorrhoeae, *colony type 4. For experimental description see Wolf-Watz and others* (1975). *Peak* 1, *lauric acid; Peak* 2, *myristic acid; Peak* 3, *pentadecanoic acid (internal standard); Peak* 4, *palmitic acid; Peak* 5, *palmitoleic acid; Peak* 6, *stearic acid; Peak* 7, *β-hydroxymyristic acid.*

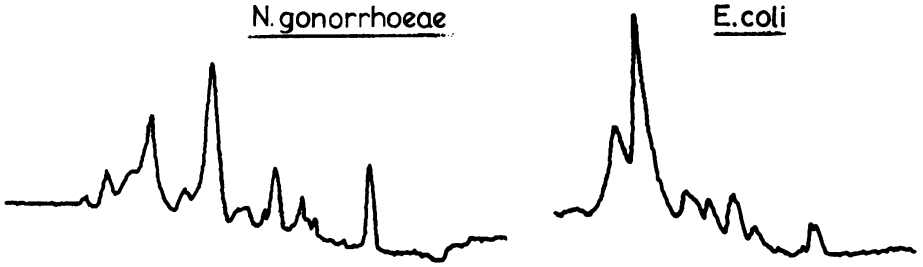

FIG. 4 *Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of OM proteins of* E. coli *K12 and* N. gonorrhoeae, *colony type* 4. *For experimental details see Wolf-Watz and others* (1975). *Migration in the gels is from right to left*

and Monner, 1975). It is tempting to suggest that this difference in primary structure is related to differences in endotoxic potential between these two species.

The main phospholipids of *E. coli* and *N. gonorrhoeae* OM are phosphatidylethanolamine, phosphatidylglycerol, and diphosphatidylglycerol. With respect to the relative distribution, no significant changes were found (Wolf-Watz and others, 1973, 1975). However, small lipid changes may markedly influence the permeability of the cell (Boman and others, 1974). Moreover, we have no absolute value for the lipid content of gonococcal OM.

The main difference between OM of *N. gonorrhoeae* and *E. coli* appears to reside in the proteins. By using polyacrylamide gel electrophoresis in sodium dodecyl sulphate roughly ten to twelve major molecular weight classes of polypeptides can be detected in *E. coli* OM. The dominating polypeptide(s) has a molecular weight of 44,000. When we subjected OM proteins of *N. gonorrhoeae* to the same treatment, about eight major polypeptide classes were found, the predominating one having a molecular weight of 35,000 (Fig. 4). It is likely, but it remains to be proved, that at least some gonococcal OM proteins are unique for this species. The significance of such proteins for possible future immunoprophylaxis and serological tests is obvious.

Johnston and Gotschlich (1974) have shown that the OM proteins of *N. gonorrhoeae* can easily be extracted in tris(hydroxymethyl)amino-methane (TRIS)-saline buffers. We have confirmed their results. However, when the OM of *E. coli* was subjected to TRIS-saline buffer no proteins were released. The OM of this species can be extracted only by the use of detergents. This would seem to imply that the OM proteins of *N. gonorrhoeae* are considerably more hydrophilic than the corresponding proteins of *E. coli*.

We argue that the passage of charged molecules (*e.g.* antibiotics) may be facilitated by the presence of hydrophilic proteins in the *N. gonorrhoeae* OM. A difference in protein solubility may well explain the marked dissimilarity in antibiotic susceptibility between *E. coli* and *N. gonorrhoeae*.

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